Trans-4-hydroxy-2-nonenal inhibits nucleotide excision repair in human cells: A possible mechanism for lipid peroxidation-induced carcinogenesis

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Lipid peroxidation (LPO) is a cellular process that commonly takes place under normal physiological conditions. Under excessive oxidative stress, the level of LPO becomes very significant, and a growing body of evidence has shown that excessive LPO may be involved in carcinogenesis. *Trans*-4-hydroxy-2-nonenal (4-HNE) is a major product of LPO, and its level becomes relatively high in cells under oxidative stress. 4-HNE is able to react readily with various cellular components, including DNA and proteins. We previously found that the 4-HNE-DNA adduct is a potent mutagen in human cells and is preferentially formed at codon 249 of the *p53* gene, a mutational hotspot in human cancers. To further understand the role of 4-HNE in carcinogenesis, we addressed the question of whether 4-HNE affects DNA repair in human cells. We found that the repair capacity for benzo[*a*]pyrene diol epoxide and UV light-induced DNA damage was greatly compromised in human cells or human cell extracts treated with 4-HNE, which is mainly through interaction of 4-HNE with cellular repair proteins. We also found that 4-HNE greatly sensitizes cells to benzo[*a*]pyrene diol epoxide- and UV-induced killing. Together these results strongly suggest that this LPO metabolite damages not only DNA but also DNA repair mechanisms in human cells. We propose that these two detrimental effects of LPO may contribute synergistically to human carcinogenesis.

Lipid peroxidation (LPO) is a common cellular process that becomes significant when cells are under oxidative stress, exposed to xenobiotics, and subjected to bacterial or viral infections (1–3). A growing body of evidence has shown that excessive LPO may play important roles in various human diseases, including carcinogenesis (2–4). The finding that individuals with a defective HFE gene have excessive amounts of iron accumulation in their liver, which consequently induces an excessive oxidative stress and a high level of LPO, is evidence that LPO may be involved in human carcinogenesis (5, 6). If preventive measures are not undertaken, these individuals develop hemochromatosis and eventually liver cancer (5). In animal models, it has also been found that a high level of LPO is tightly associated with carcinogenesis (7–10). For example, Long Evans Cinnamon rats that are defective in liver copper metabolism have elevated levels of LPO in liver (7, 8). These rats develop hepatitis at an early stage that progresses to cirrhosis, and almost all surviving rats eventually develop liver cancer (7, 8). Fisher rats fed with a choline-deficient diet or treated with carbon tetrachloride (CCl_4) also have an elevated level of LPO in their livers; these animals have a high incidence of liver cancer (9, 10). Together these results strongly suggest that the elevated level of LPO may be involved in carcinogenesis in both humans and animals.

It has been found that LPO produces many byproducts, particularly aldehydes such as acrolein, crotonaldehyde, malondialdehyde, and *trans*-4-hydroxy-2-nonenal (4-HNE) (1, 2). 4-HNE is among the most abundant and cytotoxic of these aldehydes (1, 2). Because 4-HNE contains two olefinic bonds and one carbonyl group, it reacts with not only DNA but also proteins and other molecules containing thiol groups in cells (Fig. 1) (1, 2, 11). It has been found that 4-HNE can interact with DNA to form 4-HNE-dG adduct, a bulky exocyclic DNA adduct, which has been found in various normal tissues of humans and rats (1, 9, 12). We and others (13, 14) have recently found that 4-HNE-dG adduct is a strong mutagen and induces mainly G:C to T:A mutations in human cells. We have also found that 4-HNE-dG adduct preferentially form at -GAGGC/A- sequences in the *p53* gene, including codon 249 (15), a mutational hotspot in human hepatocellular carcinoma and cigarette smoke-related lung cancer (16). Because the pathogenesis of most hepatocellular carcinoma involves hepatitis (17, 18), these cells may have a high level of LPO. It has also been found that cigarette smoke generates oxidative stress in lung cells (19, 20). These results raise the possibility that 4-HNE may play a potential important role in human carcinogenesis.

Under physiological conditions, the cellular concentration of 4-HNE ranges from 0.1 to 3 μM (1, 2). The concentration of this endogenously generated DNA-damaging agent in cells is relatively high compared to the concentrations of exogenous DNA damaging agents that cells may encounter under different environmental conditions. However, under oxidative stress conditions, 4-HNE can accumulate at even higher concentrations of 10 μM to 5 mM (1, 2). For example, in rats exposed to CCl_4 and in Long Evans Cinnamon rats, the level of 4-HNE can reach up to $\approx$100 μM in hepatocytes (7–10). It has been found that, under these conditions, not only are a significant amount of 4-HNE-DNA adducts (>100 nmol/mol of guanine) formed in the liver genome, a massive amount of 4-HNE-protein adducts are also formed (1, 2, 7, 8). It is known that 4-HNE is much more reactive to proteins than to DNA; 4-HNE can rapidly react with the sulfhydryl group of cysteine, the amino group of lysine, and the imidazole group of histidine by Michael adduction (1, 2, 11). It is very likely that many proteins involved in DNA repair may be adducted by 4-HNE, which may result in detrimental effects on cellular DNA repair capacity, and this may contribute to cytotoxicity and carcinogenicity of 4-HNE.

In this study, we investigated this possibility by determining the effect of 4-HNE on DNA repair in human cells. When host cell reactivation assay was used, we found that 4-HNE can greatly inhibit nucleotide excision repair (NER) of DNA damage induced by benzo[*a*]pyrene diol epoxide (BPDE), a major carcinogen in cigarette smoke and environment, as well as damage induced by UV light irradiation in both human colon and lung epithelial cells. The effect of 4-HNE on DNA repair was further confirmed by its inhibitory effect on DNA repair in an *in vitro* DNA repair synthesis system, and this effect is mainly caused by the direct modification of repair proteins by 4-HNE. We have also found that 4-HNE can greatly enhance the sensitivity of human cells to BPDE and UV-induced cell killing. Together, these results strongly suggest

Abbreviations: LPO, lipid peroxidation; 4-HNE, *trans*-4-hydroxy-2-nonenal; NER, nucleotide excision repair; BPDE, benzo[*a*]pyrene diol epoxide.

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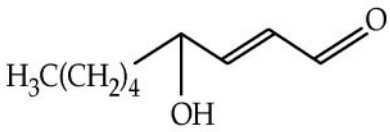

4-HNE

Fig. 1. Chemical structure of 4-HNE.

that this LPO metabolite damages not only DNA but also DNA repair mechanisms. We propose that these two detrimental effects of LPO may contribute synergistically to human carcinogenesis.

Materials and Methods

Cells and Cell Cultures. The human colon epithelial cell line HCT116 and lung epithelial A549 cells (American Type Culture Collection) were grown in McCoy's 5A medium and DMEM supplemented with 10% FBS.

Host Cell Reactivation Assay. The pGL3-luciferase plasmids (Promega) were modified with 15 μM BPDE (Chemsyn Science Laboratories, Lenexa, KS) at 25°C for 2 h or irradiated with UV light (germicidal lamp, the major emission 254 nm) at 1,500 J/m^2 as described (21–23). HCT116 and A549 cells were plated in triplicate in 60-mm dishes at a density of 3×10^5 cells per dish and exposed to various concentrations of 4-HNE (a generous gift from S. Amin, American Health Foundation, Valhalla, NY) in serum-free minimal essential medium (MEM) at 37°C for 3 h. After treatment, cells were rinsed with PBS, and then transfected with 2 μg of BPDE-modified or UV-irradiated pGL3-luciferase reporter plasmid by using FuGENE 6 transfection reagent (Boehringer Mannheim) as described (22). The untreated pSV–β-galactosidase control vector, a β-galactosidase-expressing plasmid, was cotransfected into human cells as internal control to normalize transfection efficiency. Cells were lysed with Reporter Lysis Buffer (Promega) 16 h after transfection. Transient expression of luciferase and β-galactosidase was determined as described (22). Values of luciferase expression were normalized to the β-galactosidase and averaged over the triplicates. Because the reporter gene will not express unless DNA damage induced by BPDE or UV is repaired by cells, this assay can be used to detect the repair capacity of cells. The relative luciferase activity from BPDE- or UV-treated pGL3-luciferase reporter plasmids is expressed as a percentage of luciferase activity from untreated pGL3-luciferase reporter plasmids and is used to represent the repair capacity of cells. The relative repair capacity of cells was calculated as the percentage of the relative luciferase activity of the plasmids transfected into 4-HNE-treated cells as compared to untreated cells.

Preparation of Cell Extracts. Logarithmically growing cells were treated with various concentrations of 4-HNE in serum-free MEM for 3 h at 37°C. Cells were rinsed with PBS and harvested immediately after treatment. Whole cell extracts were prepared according to the method described by Wood *et al.* (24), with the exception that the concentration of DTT in all DTT-containing buffers used for the preparation of cell extracts was 0.1 mM. The resultant cell extracts were quick-frozen in small aliquots and stored at −80°C. The protein content was determined by the Bio-Rad protein assay kit (Bio-Rad).

***In Vitro* DNA Repair Synthesis Assay.** The pUC18 and pBR322 plasmid DNA were purified by CsCl density gradient centrifugation. The supercoiled plasmid DNA was further purified by 5–20% sucrose gradients centrifugation (24, 25). The supercoiled pUC18 plasmid DNA was then modified with BPDE (15 μM) or irradiated with 1500 J/m^2 UV as described above. After modifications, the supercoiled pUC18 plasmid DNA was purified again by 5–20% sucrose gradient centrifugation.

The *in vitro* DNA repair synthesis assay (24, 25) was performed in 50-μl reaction mixtures containing 0.3 μg of supercoiled BPDE- or UV-treated pUC18 DNA, 0.3 μg of untreated supercoiled pBR322 plasmid DNA, 45 mM Hepes-KOH (pH 7.8), 70 mM KCl, 7.4 mM $MgCl_2$, 50 μM DTT, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dGTP, dCTP, and TTP, 2 μCi [α-^{32}P]dATP (3,000 Ci/mmol; 1 Ci = 37 GBq), 40 mM phosphocreatine, 2.5 μg of creatine phosphokinase (Type 1, Sigma), 3.4% glycerol, 18 μg of BSA, and 80 μg of extract protein. Reactions were carried out at 30°C for 3 h and stopped by adding EDTA (final concentration, 20 mM). The reaction mixtures were treated with 80 μg/ml RNase A for 10 min at 37°C and followed by 0.5% SDS and 0.2 mg/ml proteinase K treatment for 30 min at 37°C. Proteins in the reaction mixtures were removed by phenol/chloroform extractions, and the plasmid DNA was ethanol-precipitated, dissolved in TE buffer (10 mM Tris, pH 7.5/10 mM EDTA), and then linearized by *Hin*dIII digestion. The linearized DNA was then separated on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. The gel was first photographed, dried, and exposed to a Cyclone Phosphoimager screen (Packard). The amount of radioactive nucleotide incorporation in the DNA was quantified by a Cyclone Phosphorimger and then normalized with the DNA content measured by Bioimager. The amount of DNA repair synthesis, expressed as incorporation of [α-^{32}P]dATP per unit of DNA, was calculated by the subtraction of nonspecific incorporation measured in the undamaged control pBR322 DNA from the total incorporation measured in the damaged pUC18 DNA.

Colony-Formation Ability Assay. Logarithmically growing HCT116 and A549 cells were subjected to the following treatments: (*i*) various concentrations of 4-HNE in serum-free MEM for 3 h at 37°C, (*ii*) 0.3 μM BPDE in serum-free medium for 30 min at 37°C, (*iii*) UV irradiation (6 J/m^2), and (*iv*) 4-HNE for 3 h at 37°C, three rinses with PBS to remove 4-HNE, and then BPDE treatment or UV irradiation as described above. After these treatments, cells were rinsed with PBS, immediately trypsinized, and seeded (300 cells per dish) in fresh complete culture medium. After 9 days of incubation, colonies were fixed with methanol, stained with crystal violet, and counted (22). Colony-formation ability was calculated based on the plating efficiency of treated cells versus the plating efficiency of untreated control cells.

Results

4-HNE Inhibits Repair of BPDE- and UV-Induced DNA Damage in Human Cells. It has been found that 4-HNE forms adducts with proteins by rapidly reacting with the sulfhydryl group of cysteine, the amino group of lysine, and the imidazole group of histidine in cellular proteins (1, 2, 11); these modifications may alter protein functions, including DNA repair. To test this possibility, we determined the repair of BPDE- or UV-damaged luciferase reporter gene in 4-HNE-treated human colon epithelial cells HCT116 and lung epithelial cells A549 by using host cell reactivation assay (22, 23). We first determined the cytotoxicity of 4-HNE to HCT116 and A549 cells and found that >90% viability was observed in both types of cells at 16 h after treatment with up to 100 μM of 4-HNE for 3 h. Thus, different 4-HNE concentrations up to 100 μM were used to treat cells for 3 h in our studies to determine the effect of 4-HNE on DNA repair in human cells. The pGL3-luciferase plasmids damaged by BPDE or UV were transfected into cells with or without 4-HNE treatment. The luciferase activity was determined 16 h after transfection. Because the reporter gene will not express unless BPDE–DNA adducts or UV-induced cyclobutane pyrimidine dimers in this gene are repaired by cells, the luciferase activity therefore represents the extent of repair of BPDE–DNA

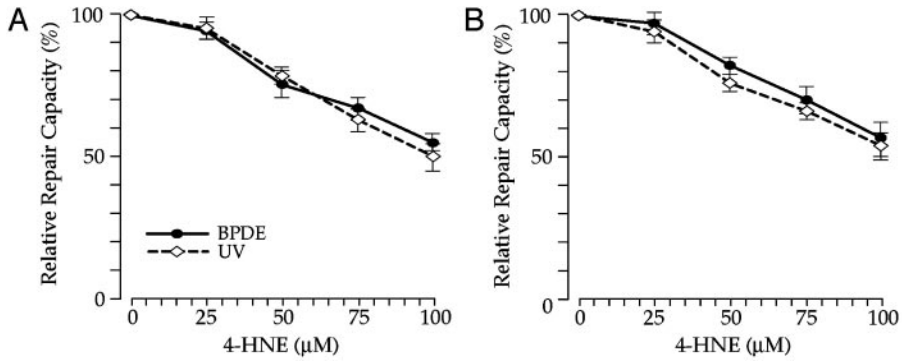

Fig. 2. 4-HNE treatment reduces cellular DNA repair capacity for BPDE- or UV-induced DNA damage. Host cell reactivation assay was performed by cotransfecting BPDE-modified or UV-irradiated pGL3-luciferase and unmodified pSV-β-galactosidase plasmids into HCT116 (*A*) and A549 (*B*) cells pretreated with different concentrations of 4-HNE (0–100 μM). The 4-HNE treatment, transfection, measurements of luciferase and β-galactosidase activities, and calculations of relative repair capacity were described in *Materials and Methods*. The data represent three independent experiments.

adducts or cyclobutane pyrimidine dimers, which in turn reflects the cellular DNA repair capacity (23). The relative repair activity detected in HCT116 cells with or without 4-HNE treatment is presented in Fig. 2*A*. Compared with cells without 4-HNE exposure, when these BPDE or UV-damaged plasmids were transfected into HCT116 cells treated with 4-HNE at the concentration of >50 μM, much lower luciferase activities were detected, which indicates that the repair capacity for BPDE–DNA adducts and cyclobutane pyrimidine dimers in 4-HNE-treated cells was greatly reduced. The extent of inhibition appears dependent on the concentrations of 4-HNE; the relative repair capacity for BPDE- and UV-induced DNA damage in cells treated with 50, 75, and 100 μM 4-HNE is decreased to ≈80%, ≈70%, and ≈50%, respectively. A similar inhibitory effect on DNA repair by 4-HNE was also observed in human lung epithelial cells; the relative repair capacity for BPDE- and UV-induced DNA damage in A549 cells treated with 50 and 100 μM 4-HNE was decreased to ≈80% and ≈60%, respectively (Fig. 2*B*).

To further demonstrate the inhibitory effect on DNA repair by 4-HNE, an *in vitro* DNA repair synthesis assay (24, 25) was performed to determine the NER repair capacity in cell extracts isolated from 4-HNE-treated HCT116 and A549 cells. Cells were treated with different concentrations of 4-HNE for 3 h, and whole cell extracts were prepared. These cell extracts were used to mediate DNA repair synthesis in the presence of [α-^{32}P]dATP using BPDE- or UV-damaged supercoiled pUC18 plasmid DNA and undamaged pBR322 plasmid DNA as substrates. Results in Fig. 3 *A* and *B* show that significant [α-^{32}P]dATP incorporation was detected only in damaged pUC18 DNA, and only background incorporation was detected in undamaged pBR322 DNA, indicating that the repair synthesis in this system is damage specific. Results in Fig. 3 demonstrate that cell extracts from 4-HNE-treated cells showed a greatly reduced repair capacity for both BPDE- and UV-induced DNA damage, and this inhibitory effect on repair depends on the concentrations of 4-HNE. Compared with the cell extracts isolated from untreated HCT116 cells, although 25 μM 4-HNE showed no significant inhibitory effect on the repair of BPDE–DNA adducts or cyclobutane pyrimidine dimers, 50 μM 4-HNE reduced the repair capacity to 70–80%, and 100 μM 4-HNE further reduced the repair capacity to 50–60% (Fig. 3 *A–C*). This 4-HNE concentration-dependent inhibitory effect on DNA repair was also observed in A549 cells as shown in Fig. 3*D*.

4-HNE Inhibits DNA Repair by Direct Modification of DNA Repair Proteins *in Vitro*. The inhibitory effect on DNA repair could be caused by adduction of repair proteins by 4-HNE altering the function of repair proteins and/or by 4-HNE treatment suppressing the expression of genes coding for repair proteins. To distinguish between these two possibilities, various concentrations of 4-HNE were added to the cell extracts isolated from 4-HNE-untreated human HCT116 and A549 cells, and these cell extracts were used to mediate *in vitro* DNA repair synthesis using BPDE- or UV-damaged pUC18 and undamaged pBR322 DNA as substrates. Results in Fig. 4 show that treating cell extract proteins with 4-HNE directly also inhibits DNA repair synthesis. Although 5 μM 4-HNE treatment did not significantly inhibit repair synthesis induced by BPDE- or UV-induced DNA damage, 50 and 100 μM 4-HNE treatment resulted in ≈50% and ≈80–90% reduction of repair synthesis, respectively. It is worth nothing that treating cell extracts directly with 4-HNE exerts more profound effect on inhibition of DNA repair synthesis than treating cells (compare Fig. 3 to Fig. 4); these results indicate that, in the latter condition, a substantial

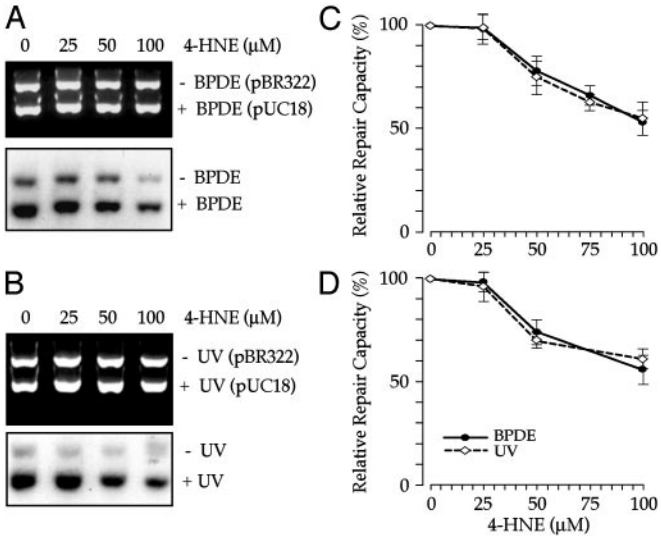

Fig. 3. 4-HNE treatment of cells inhibits *in vitro* DNA repair synthesis induced by BPDE or UV damage. Cell extracts were prepared from HCT116 (*A–C*) and A549 (*D*) cells treated with different concentrations of 4-HNE (0–100 μM). The *in vitro* DNA repair synthesis was performed by incubating BPDE- or UV-damaged pUC18 plasmids and undamaged pBR322 plasmids with cell extracts in the presence of [α-^{32}P]dATP as described in *Materials and Methods*. (*A* and *B*) Typical photographs of BPDE (*A*) and UV (*B*) damage-induced DNA repair synthesis in HCT116 cell extracts. (*Upper*) A typical photograph of ethidium bromide-stained gel. (*Lower*) An autoradiograph of the same gel. (*C* and *D*) The relative repair capacity in HCT116 and A549. The data represent three independent experiments.

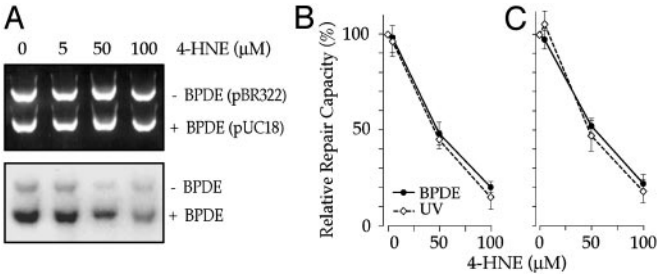

Fig. 4. DNA repair synthesis is reduced in cell extracts treated with 4-HNE directly. Cell extracts were prepared from untreated HCT116 (*A* and *B*) and A549 (*C*) cells. The *in vitro* DNA repair synthesis was performed by incubating BPDE- and UV-damaged pUC18 plasmids and undamaged pBR322 plasmids with cell extracts in the presence of different concentrations of 4-HNE (0–100 μM). (*A*) Typical photographs of BPDE damage-induced DNA repair synthesis in HCT116 cell extracts treated with 4-HNE. (*Upper*) A photograph of ethidium bromide-stained gel. (*Lower*) An autoradiograph of the same gel. (*B* and *C*) The relative repair capacity in HCT116 and A549. The data represent three independent experiments.

amount of 4-HNE may interact with components in the cell culture medium such as amino acids and/or not enter intact cells freely. These results also indicate that the inhibitory effect of 4-HNE on DNA repair observed in 4-HNE-treated cells or cell extracts is most likely caused by the direct adduction of repair proteins by 4-HNE. This conclusion is further strengthened by the results shown in Fig. 5, which demonstrate that the inhibitory effect of 4-HNE on BPDE or UV damage-induced DNA repair synthesis mediated in cell extracts from untreated HCT116 cells was greatly diminished when an excess of DTT (1 mM), the 4-HNE scavenging agent, was added into the cell extracts in addition to 4-HNE. A similar effect was also observed in A549 cell extracts (data not shown). DTT is known to be able to react with α,β-unsaturated carbonyl compounds such as 4-HNE. These results indicate that the excessive amount of DTT reacts with 4-HNE, thereby reducing the formation of 4-HNE-protein adducts and diminishing the inhibitory effect of 4-HNE on DNA repair.

4-HNE Sensitizes Human Cells to the BPDE and UV Damage-Induced Cell Killing. Results in Figs. 2–4 clearly demonstrate that 4-HNE treatment in human cells or human cell extracts can greatly reduce the cellular repair capacity for BPDE- and UV-induced DNA damage. Hence, it is possible that 4-HNE treatment may sensitize human cells to BPDE- and UV-induced cell killing. Herein, the colony-formation abilities were determined in cells treated with 4-HNE followed by BPDE or UV. 4-HNE-treated HCT116 and A549 cells (5 μM for 3 h) or untreated cells were exposed to 0.3 μM BPDE for 30 min or irradiated with UV at 6 J/m^2. Results in Table 1 show that 4-HNE pretreatment of both human cells indeed significantly enhanced BPDE or UV treatment-induced cell killing; the reduction of DNA repair capacity by 4-HNE in these human cells most likely is a major contributor to this effect.

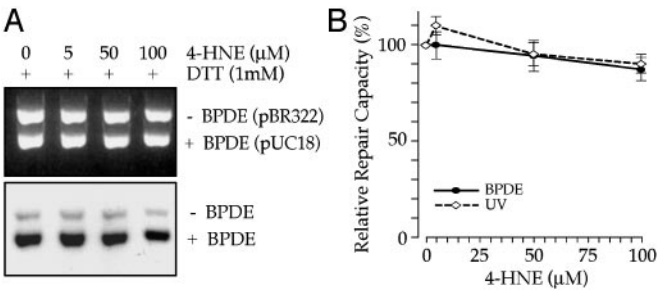

Fig. 5. DTT neutralizes the inhibitory effect of 4-HNE on BPDE or UV damage-induced DNA repair synthesis in human cell extracts. BPDE and UV damage-induced DNA repair synthesis in HCT116 cell extracts was performed as described in Fig. 4, except that 1 mM DTT was added to cell extracts along with different concentrations of 4-HNE (0–100 μM). (*A*) BPDE damage-induced DNA repair synthesis in HCT116 cell extracts treated with different concentrations of 4-HNE and 1 mM DTT. (*Upper*) A typical photograph of ethidium bromide-stained gel. (*Lower*) An autoradiograph of the same gel. (*B*) The relative repair capacity. The data represent three independent experiments.

Discussion

Oxidative stress and LPO have been suspected to be involved in many diseases, including carcinogenesis (3, 4). However, the underlying mechanisms remain unclear. In this study, we have demonstrated that 4-HNE treatment reduces cellular repair capacity for BPDE and UV-induced DNA damage. Because significant amounts of 4-HNE have been shown to widely exist in different human organs and tissues under physiological conditions, the inhibitory effect on DNA repair induced by 4-HNE may inhibit repair of various kinds of DNA damage-induced either endogenously or exogenously. We propose that this reduction of DNA repair capacity induced by LPO may contribute greatly to mutagenesis and carcinogenesis.

4-HNE is relatively stable and can pass through subcellular compartments; thus, it has the potential to interact with many different cellular proteins (1, 2). 4-HNE added exogenously to or generated endogenously in cells is able to bind to various kinds of proteins, and impairs their function (1, 2). 4-HNE-protein adducts have been detected in various tissues in a number of human diseases, such as atherosclerosis, neurodegenerative diseases, and cancers, providing evidence for the important role that the interaction of 4-HNE with proteins may play in human diseases, including carcinogenesis (1, 2). We have found that 4-HNE can inhibit NER capacity through its direct interaction with proteins involved in DNA repair. This interaction between 4-HNE and DNA repair proteins could be a major mechanism for 4-HNE-induced repair inhibition. It is possible that other mechanisms may contribute to the 4-HNE-induced repair inhibition, such as the 4-HNE modification of proteins regulating DNA repair or cofactors for repair proteins like p53 (26). It has been shown that p53 is involved in the NER pathway, and 4-HNE can impair P53 protein function through inhibition of thioredoxin reductase, a protein that governs normal p53 conformation and function (27). Alteration of signal transduction pathways that may regulate DNA damage recognition and repair may be another mechanism that contributes to 4-HNE-induced repair inhibition. It has been shown that 4-HNE can activate many signal transduction pathways, such as the mitogen-activated protein kinase (MAPK), PKC, and NF-κB pathways, and displays various biological functions (2, 28, 29). Finally, 4-HNE may affect the gene expression of repair proteins. Although 4-HNE has

Table 1. Effect of 4-HNE treatment on colony-formation ability of HCT116 and A549 cells treated with BPDE or UV

Treatment			Colony-formation ability, %	
4-HNE,* μM	BPDE,* μM	UV, J/m^2	HCT116	A549
0	0	0	100	100
5	0	0	93 ± 7	89 ± 6
0	0.3	0	56 ± 5	63 ± 5
5†	0.3	0	25 ± 3	36 ± 4
0	0	6	58 ± 4	61 ± 5
5‡	0	6	28 ± 2	37 ± 3

*Exposure time to 4-HNE was 3 h, and exposure time to BPDE was 30 min.
†Cells were treated with 4-HNE followed by BPDE.
‡Cells were treated with 4-HNE followed by UV.

been shown to alter gene expression in human cells, it does not change the expression of repair genes at a significant level (30).

It is well established that DNA damage and repair play important roles in human disease processes, including carcinogenesis, and NER is the most important repair pathway to repair various kinds of bulky DNA adducts (31, 32). Individuals who have genetic defects in NER repair genes such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS) genes develop neural and immunological diseases (31–33). Most of XP patients eventually develop skin cancer, and CS patients suffer many abnormalities and have a much shorter lifespan (31–33). It has been shown that lung cancer patients have much lower repair capacities for BPDE-induced DNA damage (34). These results are consistent with the hypothesis that compromised DNA repair capacity may increase susceptibility to cancer. These results also raise the possibility that, if the repair capacity of individuals is compromised by agents generated endogenously or present in the environment, these individuals, even if they have normal repair genes, may have higher susceptibility to various diseases including cancer. It has been found that XPA and XPC patients who suffer >80 and 98% reduction of NER capacity have a 2,000-fold increase in skin cancer incidence (35, 36). Based on these results, we speculate that the organs of individuals under constant oxidative stress such as the liver of hepatitis B patients may have a greatly elevated possibility to develop cancer, particularly, when they are constantly exposed to DNA damaging agents such as aflatoxin B1. These individuals will be under similar conditions as XP patients: they not only may have reduced NER capacity but also are constantly exposed to DNA damaging agents. It should be noted that LPO generates substantial amount of aldehydes other than 4-HNE, and these aldehydes may also inhibit other repair pathways such as base excision and mismatch repair. If this is the case, then LPO may have even more profound effects on human carcinogenesis.

Previously, we and other (13, 14) have found that the 4-HNE-dG adduct induces mainly G:C to T:A transversions in human cells, and 4-HNE forms DNA adducts preferentially at codon 249 of *p53* gene (15), a mutational hotspot in hepatocellular carcinoma and smoke-related lung cancer (16). Recently, it has been shown that 4-HNE can indeed induce a high frequency of G:C to T:A mutations at codon 249 of the *p53* gene (37). It is possible that the level of 4-HNE necessary to produce significant amounts of binding at codon 249 also greatly compromises cellular DNA repair capacity. These two effects of 4-HNE probably greatly enhance the chance of 4-HNE to induce mutations at codon 249 of the *p53* gene. It has been found that hepatocytes with a mutation at codon 249 gain a growth advantage and are more resistant to apoptosis, and P53 proteins with this mutation manifest a dominant-negative effect on transcription transactivation (38). These factors together may be an important reason why codon 249 of *p53* gene is a mutational hotspot in hepatocellular carcinoma. Cigarette smoke is the major cause of lung cancer, and polycyclic aromatic hydrocarbons generated in cigarette smoke have been suggested as being responsible for the initiation and development of lung cancer (39). Previously, we have found that polycyclic aromatic hydrocarbons including BPDE preferentially bind at mutational hotspots in smoke-related lung cancer, such as codons 157, 158, 245, 248, 273, and 282, but not codon 249 (16, 40). These findings raise the possibility that carcinogens other than polycyclic aromatic hydrocarbons may cause mutations at codon 249. It has been found that cigarette smoke generates oxidative stress in lung cells (19, 20); perhaps LPO metabolites such as 4-HNE generated in lung cells may cause mutations at codon 249 of the *p53* gene in cigarette smoke-related lung cancer.

In summary, in this study we have demonstrated that 4-HNE can greatly inhibit DNA repair capacity in human cells through its direct interaction with repair proteins. It is likely that other aldehydes, such as acrolein, crotonaldehyde, and malondialdehyde, also manifest a similar effect. The inhibition of DNA repair by LPO metabolites may prove to be a major cause of LPO-induced human diseases, including carcinogenesis.

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